REMARKS

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the foregoing amendments and following remarks, and the accompanying publically-available database printouts. Applicant's representative thanks the Examiner for indicating the withdrawal of many of the previously made rejections. The foregoing amendments are fully supported by the specification, particularly at paragraph [0029] and in the sequence listing, and no new matter is added.

Compliance with the Sequence Rules

In the Office Action at paragraph 6, the Examiner has noted that the statement submitted on February 15, 2005 failed to affirm that no new matter is included in the CRF. A signed statement regarding no new matter has been submitted herewith. Applicants greatly appreciate the Examiner's pointing out this oversight.

Withdrawal of Previous Rejections

In the Office Action at paragraph 11, the Examiner has withdrawn the previous rejection of claims 1-5 under 35 U.S.C. §112, 2nd paragraph. However, the Examiner states in her reasons for withdrawal that the Examiner understands that the term "major" in the phrase "major carbon source" means the predominant, i.e. "major" means that the carbon source is the predominant source. Such definition is based upon applicant's arguments presented in the response filed February 15, 2005 on page 10. Although the Examiner's interpretation appears to be correct, to further clarify the record, the specification states at paragraph [0029] that "the methanol-assimilating bacterium, that is, methylotroph, means a bacterium which can grow by utilizing methanol as a major carbon source". This statement cannot mean anything except that **methanol** is the "major" or "predominant" source of carbon in the medium that is utilized by the

bacterium for growth. Therefore, when the Examiner states that "the carbon source is the predominant source", the record should reflect that the predominant or major source of carbon is methanol, as clearly reflected by the above-statement in the specification.

Rejection under 35 U.S.C. § 112, first paragraph

In the Office Action, beginning at page 6, the rejection to Claims 2-4 and 6-7 under 35 U.S.C. § 112, first paragraph, was maintained as allegedly lacking enablement. Applicant respectfully requests reconsideration of this rejection.

In a telephone interview with the Examiner on September 1, 2005, possible additional data that might be submitted to further support the arguments made in the previous response of February 15, 2005 was discussed. The Examiner suggested more alignment data showing the similarity of the LysE protein of *Corynebacterium glutamicum* (SEQ ID NO: 2) with other diverse LysE proteins from other bacteria, in order to demonstrate that one of ordinary skill in the art would be able to routinely determine substitutions, deletions, or insertions that might be made in the protein of SEQ ID NO:2 without changing the ability to impart resistance to S-(2-aminoethyl) cysteine when introduced into said methylotroph. Applicant's representative greatly appreciates the suggestions provided by the Examiner, and the following presentation of data and arguments result directly from these suggestions.

First, applicants hereby submit alignment data of LysE protein of *Coynebacterium glutamicum* (SEQ ID NO:2) and YggA protein of *E. coli* (Appendix A). The YggA protein is a putative amino acid transport protein which shares similarity with LysE protein of *Coynebacterium glutamicum*. It is noted that the YggA protein is registered as NP_417398 with a definition of "LysE family" in the protein database of NCBI, as shown in pages 2-3 of Appendix A. The alignment data shows that the YggA protein has Gly at position 57, which is presumed to correspond to Gly at position 56 of the LysE protein. This data also shows which positions are conserved and which are not between

these two proteins from diverse bacteria, and therefore provides ample and sufficient guidance as to which positions might be tolerant to substitution, deletion, or insertion of amino acids while maintaining the claimed activity of imparting resistance to S-(2-aminoethyl) cysteine when introduced into a methanol-assimilating bacteria.

Secondly, applicants hereby submit alignment data of the LysE protein of *Coynebacterium glutamicum* (SEQ ID NO:2) and *Coynebacterium diphtheriae*, which shows that Gly at position 56 is also conserved in the amino acid sequence of the LysE protein of *Coynebacterium diphtheriae* (page 4 of Appendix A). For the sequence information of the LysE protein of *Coynebacterium diphtheriae*, please refer to pages 5-6 of APPENDIX A. This data presents another example of an alignment of two lysine exporter proteins, and which shows positions which are conserved and which are not, and therefore further provides additional guidance as to which positions might be tolerant to substitution, deletion, or insertion of amino acids while maintaining the claimed activity of imparting resistance to S-(2-aminoethyl) cysteine when introduced into said methylotroph.

Thirdly, applicants submit an alignment of the claimed lysE protein (SEQ ID NO: 2) with the lysE protein from *Corynebacterium efficiens* (see pages 7-9 of Appendix A). This data provides even further evidence of the sequence characteristics of another lysE protein, and hence provides even further information to the skilled art worker as to which positions might be tolerant to substitution, deletion, or insertion of amino acids while maintaining the claimed activity of imparting resistance to S-(2-aminoethyl) cysteine when introduced into said methylotroph.

This alignment data between LysE depicted in SEQ ID NO: 2 and lysE transporter-type proteins from *E. coli, Corynebacterium diphtheriae*, and *Corynebacterium efficiens* clearly show that one of ordinary skill in the art would be enabled to practice the claimed invention without undue experimentation, since lysE transporter proteins from other bacteria, even one as diverse as *E. coli*, were known, and

such sequence information clearly would enable the skilled art worker to make or allow for variations to the sequence of up to 10 amino acids different from the sequence shown in SEQ ID NO: 2 while maintaining the ability to impart resistance to S-(2-aminoethyl) cysteine when introduced into said methylotroph.

For at least the foregoing reasons, Applicant respectfully submits that Claims 2-4 and 6-7 fully comply with 35 U.S.C. § 112, first paragraph, and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 112.

Rejection under 35 U.S.C. § 112, second paragraph

In the Office Action, beginning at page 7, Claim 2 was rejected under 35 U.S.C. § 112, second paragraph, as reciting subject matters that allegedly are indefinite. Applicant respectfully requests reconsideration of this rejection.

The claims have been amended as suggested by the Examiner, and the antecedents have been corrected. Therefore, for at least the foregoing reasons, Applicant respectfully submits that Claim 2 fully complies with 35 U.S.C. § 112, second paragraph, and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 112.

Conclusion

For at least the foregoing reasons, Applicant respectfully submits that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Kerr believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, she is invited to call on the number below.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the undersigned authorizes the charging of such fees to our deposit account 50-2821.

Respectfully submitted,

By: _____
Shelly Guest Cermak
Registration No. 39,571

U.S. P.T.O. Customer No. 38108
Cermak & Kenealy, LLP
515 E. Braddock Road, Suite B
Alexandria, VA 22314
703.778.6608

Date: September 20, 2005

APPENDIX A

Sequence similarity between the LysE protein from *Corynebacterium glutamicum* and the YggA protein from *Escherichia coli*

Glycine residue

```
LysE(Corynebacterium glutamicum).prj   1:MEIF-ITGLLLGASLLLSIGPQNVLVIKQGIKREGLIAVLLVCLISDVFLFIAGTLGVDL-LSNAAPIVL 68
yggA(Escherichia coli).prj             1:MPSYYFQGLALGAAMILPLGPQNAFVMNQGIRRQYHIMIALLCAISDLVLICAGIFGGSALLMQS-PWLL 69
                                          *      ** ***   *   ****  *  *** *   *    * * ***  *  **   *     *   * *

LysE(Corynebacterium glutamicum).prj  69:DIMRWGGIAYLLWFAVMAAKDAMTNKVEAPQIIEETEPTVPDDTPLGGSAVATDTRNRVRVEVSVDKQRV 138
yggA(Escherichia coli).prj            70:ALVTWGGVAFLLWYGFGAFKTAMSSNI------------------ELASAEVMKQG--------------R 108
                                            *** * ***    * * **                  *    *

LysE(Corynebacterium glutamicum).prj 139:W--VKPMLMAIVLTWLNPNAYLDAFVFIGGVGAQYGDTGR-WIFAAGAFAASLIWFP-LVG-FGAAALSR 203
yggA(Escherichia coli).prj           109:WKIIATMLA-V--TWLNPHVYLDTFVVLGSLGGQLDVEPKRW-FALGTISASFLWFFGL-ALLA-AWLAP 172
                                          *      **     *****  *** **   *   * *        * ** *    **  **  *      * *

LysE(Corynebacterium glutamicum).prj 204:PLSSPKVWRWINVVVAVV--MTALAIK--------LMLMG                              233
yggA(Escherichia coli).prj           173:RLRTAKAQRIINLVVGCVMWFIALQLARDGIAHAQA-LFS                              211
                                           *    *   * ** **   *     **         *
```

1: NP_417398. Reports putative amino ac...[gi:16130824]

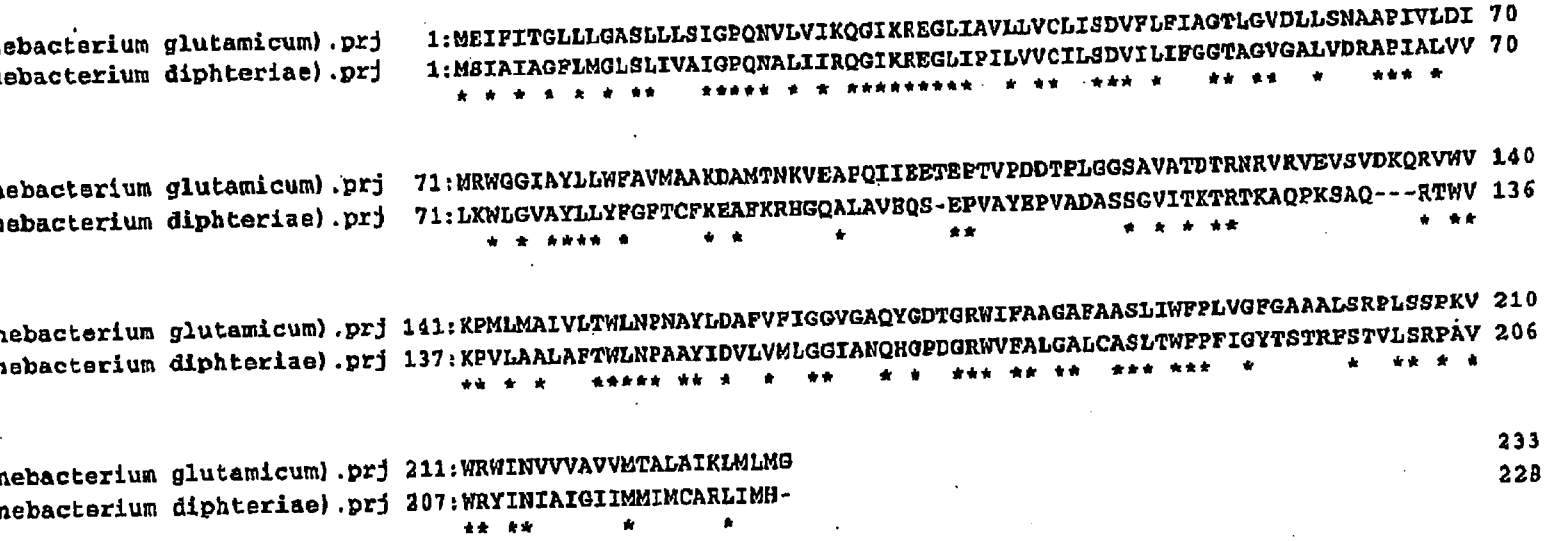
LOCUS NP_417398 211 aa linear BCT 02-SEP-2005
DEFINITION putative amino acid transport protein (LYSE family) [Escherichia coli K12].
ACCESSION NP_417398
VERSION NP_417398.1 GI:16130824
DBSOURCE REFSEQ: accession NC_000913.2
KEYWORDS .
SOURCE Escherichia coli K12
ORGANISM Escherichia coli K12
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia.
REFERENCE 1 (residues 1 to 211)
AUTHORS Aleshin,V.V., Zakataeva,N.P. and Livshits,V.A.
TITLE A new family of amino-acid-efflux proteins
JOURNAL Trends Biochem. Sci. 24 (4), 133-135 (1999)
PUBMED 10322417
REFERENCE 2 (residues 1 to 211)
AUTHORS Blattner,F.R., Plunkett,G. III, Bloch,C.A., Perna,N.T., Burland,V., Riley,M., Collado-Vides,J., Glasner,J.D., Rode,C.K., Mayhew,G.F., Gregor,J., Davis,N.W., Kirkpatrick,H.A., Goeden,M.A., Rose,D.J., Mau,B. and Shao,Y.
TITLE The complete genome sequence of Escherichia coli K-12
JOURNAL Science 277 (5331), 1453-1474 (1997)
PUBMED 9278503
REFERENCE 3 (residues 1 to 211)
AUTHORS Arnaud,M., Berlyn,M.K.B., Blattner,F.R., Galperin,M.Y., Glasner,J.D., Horiuchi,T., Kosuge,T., Mori,H., Perna,N.T., Plunkett,G. III, Riley,M., Rudd,K.E., Serres,M.H., Thomas,G.H. and Wanner,B.L.
TITLE Workshop on Annotation of Escherichia coli K-12
JOURNAL Unpublished
REMARK Woods Hole, Mass., on 14-18 November 2003 (sequence corrections)
REFERENCE 4 (residues 1 to 211)
AUTHORS Glasner,J.D., Perna,N.T., Plunkett,G. III, Anderson,B.D., Bockhorst,J., Hu,J.C., Riley,M., Rudd,K.E. and Serres,M.H.
TITLE ASAP: Escherichia coli K-12 strain MG1655 version m56
JOURNAL Unpublished
REMARK ASAP download 10 June 2004 (annotation updates)
REFERENCE 5 (residues 1 to 211)
AUTHORS Hayashi,K., Morooka,N., Mori,H. and Horiuchi,T.
TITLE A more accurate sequence comparison between genomes of Escherichia coli K12 W3110 and MG1655 strains
JOURNAL Unpublished
REMARK GenBank accessions AG613214 to AG613378 (sequence corrections)
REFERENCE 6 (residues 1 to 211)
AUTHORS Perna,N.T.

[*]

```
  REMARK     GenBank accession AY605712 (sequence corrections)
REFERENCE    7  (residues 1 to 211)
  AUTHORS    .
  CONSRTM    NCBI Genome Project
  TITLE      Direct Submission
  JOURNAL    Submitted (10-SEP-2004) National Center for Biotechnology
             Information, NIH, Bethesda, MD 20894, USA
REFERENCE    8  (residues 1 to 211)
  AUTHORS    Blattner,F.R. and Plunkett,G. III.
  TITLE      Direct Submission
  JOURNAL    Submitted (10-JUN-2004) Laboratory of Genetics, University of
             Wisconsin, 445 Henry Mall, Madison, WI 53706, USA
  REMARK     Sequence update by submitter
REFERENCE    9  (residues 1 to 211)
  AUTHORS    Plunkett,G. III.
  TITLE      Direct Submission
  JOURNAL    Submitted (13-OCT-1998) Laboratory of Genetics, University of
             Wisconsin, 445 Henry Mall, Madison, WI 53706, USA
REFERENCE    10 (residues 1 to 211)
  AUTHORS    Blattner,F.R. and Plunkett,G. III.
  TITLE      Direct Submission
  JOURNAL    Submitted (02-SEP-1997) Laboratory of Genetics, University of
             Wisconsin, 445 Henry Mall, Madison, WI 53706, USA
REFERENCE    11 (residues 1 to 211)
  AUTHORS    Blattner,F.R. and Plunkett,G. III.
  TITLE      Direct Submission
  JOURNAL    Submitted (16-JAN-1997) Laboratory of Genetics, University of
             Wisconsin, 445 Henry Mall, Madison, WI 53706, USA
COMMENT      PROVISIONAL REFSEQ: This record has not yet been subject to final
             NCBI review. The reference sequence was derived from AAC75960.
             Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..211
                     /organism="Escherichia coli K12"
                     /strain="K-12"
                     /sub_strain="MG1655"
                     /db_xref="taxon:83333"
     Protein         1..211
                     /product="putative amino acid transport protein (LYSE
                     family)"
                     /function="orf; Unknown"
     CDS             1..211
                     /gene="yggA"
                     /locus_tag="b2923"
                     /coded_by="complement(NC_000913.2:3066195..3066830)"
                     /transl_table=11
                     /db_xref="ASAP:9591"
                     /db_xref="ECOCYC:EG11159"
                     /db_xref="GeneID:947418"
ORIGIN
        1 mfsyyfqgla lgaamilplg pqnafvmnqg irrqyhimia llcaisdlvl icagifggsa
       61 llmqspwlla lvtwggvafl lwygfgafkt amssnielas aevmkqgrwk iiatmlavtw
      121 lnphvyldtf vvlgslggql dvepkrwfal gtisasflwf fglallaawl aprlrtakaq
      181 riinlvvgcv mwfialqlar dgiahaqalf s
//
```

Sequence similarity between lysine exporter proteins from *Corynebacterium glutamicum* and *Corynebacterium diphtheriae*

Glycine residue



1: NP_939452. Reports lysine exporter p...[gi:38233685]

```
LOCUS       NP_939452                 228 aa            linear   BCT 12-NOV-2004
DEFINITION  lysine exporter protein [Corynebacterium diphtheriae NCTC 13129].
ACCESSION   NP_939452
VERSION     NP_939452.1  GI:38233685
DBSOURCE    REFSEQ: accession NC_002935.2
KEYWORDS    complete genome.
SOURCE      Corynebacterium diphtheriae NCTC 13129
  ORGANISM  Corynebacterium diphtheriae NCTC 13129
            Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
            Corynebacterineae; Corynebacteriaceae; Corynebacterium.
REFERENCE   1  (residues 1 to 228)
  AUTHORS   Cerdeno-Tarraga,A.M., Efstratiou,A., Dover,L.G., Holden,M.T.G.,
            Pallen,M., Bentley,S.D., Besra,G.S., Churcher,C., James,K.D., De
            Zoysa,A., Chillingworth,T., Cronin,A., Dowd,L., Feltwell,T.,
            Hamlin,N., Holroyd,S., Jagels,K., Moule,S., Quail,M.A.,
            Rabbinowitsch,E., Rutherford,K., Thomson,N.R., Unwin,L.,
            Whitehead,S. and Barrell B.G. Parkhill,J.
  TITLE     The complete genome sequence and analysis of Corynebacterium
            diphtheriae NCTC13129
  JOURNAL   Nucleic Acids Res. 31 (22), 6516-6523 (2003)
   PUBMED   14602910
REFERENCE   2  (residues 1 to 228)
  AUTHORS   Cerdeno-Tarraga,A.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-OCT-2003) Cerdeno-Tarraga A.M., submitted on behalf
            of the Pathogen Sequencing Unit, Sanger Institute, Wellcome Trust
            Genome Campus, Hinxton, Cambridge CB10 1SA E-mail:
            amct@sanger.ac.uk
REFERENCE   3  (residues 1 to 228)
  AUTHORS   .
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (08-APR-2002) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from CAE49614.
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..228
                     /organism="Corynebacterium diphtheriae NCTC 13129"
                     /strain="NCTC13129"
                     /db_xref="taxon:257309"
                     /note="biotype gravis"
     Protein         1..228
                     /product="lysine exporter protein"
     CDS             1..228
```

[*]

```
                     /note="Similar to Corynebacterium glutamicum lysine
                     exporter protein LysE SW:LYSE_CORGL (P94633) (233 aa)
                     fasta scores: E(): 3.8e-40, 45.02% id in 231 aa, and to
                     Escherichia coli hypothetical protein YggA or B2923
                     SW:YGGA_ECOLI (P11667) (211 aa) fasta scores: E():
                     3.1e-09, 32.44% id in 225 aa"
                     /transl_table=11
                     /db_xref="GeneID:2650833"
ORIGIN
        1 msiaiagflm glslivaigp qnaliirqgi kreglipilv vcilsdvili fggtagvgal
       61 vdrapialvv lkwlgvayll yfgftcfkea fkrhgqalav eqsepvayep vadassgvit
      121 ktrtkaqpks aqrtwvkpvl aalaftwlnp aayidvlvml ggianqhgpd grwvfalgal
      181 casltwfpfi gytstrfstv lsrpavwryi niaigiimmi mcarlimh
//
```

Table 1

Sequence similarity of lysine exporter proteins from *Corynebacterium glutamicum* and *Corynebacterium efficiens*

Gly 56

```
lysE(Corynebacterium efficiens).prj    1:MEIFVTGLLLGASLLLAIGPQNVLVIKQGIKREGITAVIIVCLLSDVVLFTLGTLGVGLISDTAPIILDI 70
LysE(Corynebacterium glutamicum).prj   1:MEIFITGLLLGASLLLSIGPQNVLVIKQGIKREGLIAVLLVCLISDVFLFIAGTLGVDLLSNAAPIVLDI 70
                                         **** *********** ****************  **  *** *** **  ***** * *  *** ***

lysE(Corynebacterium efficiens).prj    71:LRWCGIAYLLWFAVMAARDALRARTEVTFV-EHSEPVAAASASGGGVTTK-Q-RPRLRITSGTR-Q-VNV 135
LysE(Corynebacterium glutamicum).prj   71:MRWGGIAYLLWFAVMAAKDAMTNKVEAPQIIEETEPTVPDDTPLGGSAVATDTRNRVRVEVSVDKQRVWV 140
                                         ** ************** **     *    * **        **       * * *       * ***

lysE(Corynebacterium efficiens).prj    136:RPMLMAIVLTWLNPNAYLDAFVFIGGVGAQYGETGRWIFAAGAFAASLVWFPLVGYGAAALSRPLSSPRV 205
LysE(Corynebacterium glutamicum).prj   141:KPMLMAIVLTWLNPNAYLDAFVFIGGVGAQYGDTGRWIFAAGAFAASLIWFPLVGFGAAALSRPLSSPKV 210
                                         ******************************** *************** ****** ************ *

                                                                                                                228
lysE(Corynebacterium efficiens).prj    206:WRWINIGVAVVLTGLAVKLILMG                                               233
LysE(Corynebacterium glutamicum).prj   211:WRWINVVVAVVMTALAIKLMLMG
                                         *****  **** * ** ** ***
```

```
LOCUS       NP_737967                 235 aa            linear   BCT 10-MAR-2005
DEFINITION  lysine exporter protein [Corynebacterium efficiens YS-314).
ACCESSION   NP_737967
VERSION     NP_737967.1  GI:25027913
DBSOURCE    REFSEQ: accession NC_004369.1
KEYWORDS    .
SOURCE      Corynebacterium efficiens YS-314
  ORGANISM  Corynebacterium efficiens YS-314
            Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
            Corynebacterineae; Corynebacteriaceae; Corynebacterium.
REFERENCE   1  (residues 1 to 235)
  AUTHORS   Nishio,Y., Nakamura,Y., Kawarabayasi,Y., Usuda,Y., Kimura,E.,
            Sugimoto,S., Matsui,K., Yamagishi,A., Kikuchi,H., Ikeo,K. and
            Gojobori,T.
  TITLE     Comparative complete genome sequence analysis of the amino acid
            replacements responsible for the thermostability of Corynebacterium
            efficiens
  JOURNAL   Genome Res. 13 (7), 1572-1579 (2003)
  PUBMED    12840036
REFERENCE   2  (residues 1 to 235)
  AUTHORS   .
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (15-NOV-2002) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   3  (residues 1 to 235)
  AUTHORS   Kawarabayasi,Y., Yamazaki,J., Hino,Y., Kikuchi,H. and
            Director-General of Biotechnology Center.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-MAY-2002) Director-General of Biotechnology Center,
            National Institute of Technology and Evaluation, Biotechnology
            Center; Nishihara 2-49-10, Shibuya-ku, Tokyo 151-0066, Japan
            (E-mail:bio@nite.go.jp, Tel:81-3-3481-1933, Fax:81-3-3481-8424)
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from BAC18167.
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..235
                     /organism="Corynebacterium efficiens YS-314"
                     /db_xref="taxon:196164"
     Protein         1..235
                     /product="lysine exporter protein"
     CDS             1..235
                     /gene="lysE"
                     /locus_tag="CE1357"
                     /coded_by="complement(NC_004369.1:1422769..1423476)"
                     /note="similar to X96471-2|CAA65324.2| percent identity:
                     71 in 228 aa"
                     /transl_table=11
                     /db_xref="GeneID:1031976"
ORIGIN
        1 mcanmrdmei fvtglllgas lllaigpqnv lvikqgikre gitaviivcl lsdvvlftlg
       61 tlgvglisdt apiildilrw cgiayllwfa vmaardalra rtevtfvehs epvaaasasg
```

```
121 ggvttkqrpr lritsgtrqv wvrpmlmaiv ltwlopnayl dafvfiggvg aqygetgrwi
181 faagafaasl vwfplvgyga aalsrplesp rvwrwinigv avvltglavk lilmg
//
```


Sep 9 2005 14:51:10